PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006805	18.06.2004	20.06.2003

International Patent Classification (IPC) or both national classification and IPC
C12N9/50, C07K14/81

Applicant
NESTEC S.A.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Page, M

Telephone No. +49 89 2399-7322

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/006805

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☐ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 9-12 (completely) and 17-23 (partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 9-12 (completely) and 17-23 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-8, 13-16 (completely) and 17-23 (partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4, 13-16 (completely), 17-23 (partially)
	No:	Claims	5-8 (completely) 17-22 (partially)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8, 13-16 (completely), 17-23 (partially)
Industrial applicability (IA)	Yes:	Claims	1-8, 13-16 (completely), 17-23 (partially)
	No:	Claims	

2. Citations and explanations

see separate sheet

The application concerns the provision of eight separate polypeptides from the coffee plant. The application ascribes protease or protease inhibitor activity to these proteins, although it is not clear how the Applicant arrives at this assumption, as neither chemical tests nor functional sequence alignments are provided. The intention of the application is to influence coffee flavour through manipulation of the genes encoding these proteins.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

III.1 The Applicant has elected not to pay additional search fees for Invention III as defined below under IV.2 (claims 9-12, pertaining to a polypeptide corresponding to SEQ ID NO. 6 or 8). As a result, no opinion regarding novelty or inventive step can be rendered for this subject matter.

Re Item IV

Lack of Unity of Invention

IV.1 The application seeks protection for eight separate polypeptides and their corresponding polynucleotides. Two of the polypeptides have been nominated as cysteine proteinases (SEQ ID NOs. 2 and 16), four as cysteine proteinase inhibitors (SEQ ID NO. 4, 10, 12 and 16) and two are apparently related aspartic endoproteinases (SEQ ID NOs. 6 and 8). There is no technical feature disclosed in the application that unites these polypeptides in a manner that elevates them as a group over the prior art. The group of polypeptides does not have any discernable defining structural features that would distinguish them from sequences already known in the art. In fact certain sequences are more closely related to sequences already known in the art than to each other (see e.g. Uniprot accession number Q9ARH0, 73% identical to SEQ ID NO. 10 over its entire length).

IV.2 The requirements of Rule 13.1 PCT are therefore not met and the application is divided up into the following four distinct inventions:

Invention I (claims 1-4 (completely), 17-23 (partially))
Polynucleotide encoding SEQ ID NO. 2 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Invention II (claims 5-8 (completely), 17-23 (partially))
Polynucleotide encoding SEQ ID NO. 4, 10, 12 or 14 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Invention III (claims 9-12 (completely), 17-23 (partially))
Polynucleotide encoding SEQ ID NO. 6 or 8 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Invention IV (claims 13-16 (completely), 17-23 (partially))
Polynucleotide encoding SEQ ID NO. 16 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 Reference is made to the following documents:

D1: DATABASE EMBL [Online] 29 April 1994 (1994-04-29), NONG, V. ET AL.: XP002269560 retrieved from EBI accession no. EMBL Database accession no. Z32795

D2: DATABASE UNIPROT [Online] 1 March 2002 (2002-03-01), YAMADA, K. ET AL.: XP002269561 retrieved from EBI accession no. UNIPRT Database accession no. Q8VYS0

D3: MARRACCINI PIERRE ET AL: "Molecular cloning of the complete 11S seed storage protein gene of Coffea arabica and promoter analysis in transgenic tobacco plants" PLANT PHYSIOLOGY AND BIOCHEMISTRY, GAUTHIER-VILLARS, PARIS, FR, vol. 37, no. 4, April 1999 (1999-04), pages 273-282, XP002197483 ISSN: 0981-9428

D4: LEROY T ET AL: "GENETICALLY MODIFIED COFFEE PLANTS EXPRESSING

THE BACILLUS THURINGIENSIS CRY1AC GENE FOR RESISTANCE TO LEAF MINER" PLANT CELL REPORTS, SPRINGER VERLAG, DE, vol. 19, no. 4, 2000, pages 382-389, XP001002322 ISSN: 0721-7714

D5: WO 02/04617 A (KOCHHAR SUNIL ;NESTLE SA (CH); BUCHELI PETER (FR); LALOI MARYSE (F) 17 January 2002 (2002-01-17)

D6: WO 02/42327 A (KOCHHAR SUNIL ;NESTLE SA (CH); HANSEN CARL ERIK (CH); JUILLERAT MA) 30 May 2002 (2002-05-30)

D7: LING J-Q ET AL: "Cloning of two cysteine proteinase genes, CysP1 and CysP2, from soybean cotyledons by cDNA representational difference analysis" BIOCHIMICA ET BIOPHYSICA ACTA . GENE STRUCTURE AND EXPRESSION, ELSEVIER, AMSTERDAM, NL, vol. 1627, no. 2-3, 19 June 2003 (2003-06-19), pages 129-139, XP004431612 ISSN: 0167-4781

D8: DATABASE USPTO Proteins [Online] 14 February 2001 (2001-02-14), "Sequence 74 from patent US 6103514." XP002310749 retrieved from EBI accession no. USPOP:AAE48221 Database accession no. AAE48221

D9: DATABASE Geneseq [Online] 17 October 2000 (2000-10-17), "Arabidopsis thaliana protein fragment SEQ ID NO: 36701." XP002310750 retrieved from EBI accession no. GSN:AAG30665 Database accession no. AAG30665

V.2 Novelty - Art.33(1) and (2) PCT:

2.1 Invention I (claims 1-4 (completely), 17-23 (partially))

The subject matter of **claims 1-4** appears to be novel in light of the available prior art. The sequences disclosed at the time of filing with the highest degree of identity to those of the application are as follows:

SEQ ID NO.	Length	Prior Art	Length	% Sequence Identity	Overlap
1	1543 nt	D1: Z32795	1441 nt	75.5%	702 nt
2	397 aa	D2: Q8VYS0	367 aa	70.1%	380 aa
2	397 aa	D9: AAG30665	277 aa	73.8%	287 aa

2.2 **Invention II (claims 5-8 (completely), 17-23 (partially))**

The subject matter of **claims 5-8** is not novel in light of D7. Sequences 70% or more identical to SEQ ID NO. 10 are anticipated by the sequence Q9ARH0, a soybean cysteine proteinase.

SEQ ID NO.	Length	Prior Art	% Sequence Identity	Overlap (parent:prior art)
4	139 aa	⌀	-	-
10	98 aa	Q8VX72	72.2%	1-90:1-90
10	98 aa	Q9ARH0 (D7)	73.2%	1-98:1-98
10	98 aa	Q9M4Q4	68.4%	1-98:1-101
12	124 aa	⌀	-	-
14	119 aa	⌀	-	-

⌀ - no relevant sequence found

2.3 **Invention IV (claims 13-16 (completely), 17-23 (partially))**

The subject matter of **claims 13-16** appears to be novel in light of the available prior art. The sequences disclosed at the time of filing with the highest degree of identity to those of the application are as follows:

SEQ ID NO.	Length	Prior Art	% Sequence Identity	Overlap (parent:prior art)
16	359 aa	Q7X750	70.6%	1-359:1-362
16	359 aa	CYSP_VIGMU	69.8%	1-359:1-362
16	359 aa	AAE48221 (D8)	69.8%	1-359:1-362

2.4 None of the anticipated sequences are disclosed as being used in modulating coffee flavour. Accordingly, **claims 1-4, 13-16 and 23** appear to be novel in light of the cited prior art.

V.3 Inventive Step - Art.33(1) and (3) PCT:

3.1 Although the cited prior art does not disclose a solution to the technical problem of modulating coffee flavour precursor levels, the application is not considered to have demonstrated inventive step. The Applicant states on page 3 of the description that differences exist in the levels and amounts of the major storage proteins in green coffee and that small differences exist between the storage proteins of immature and mature coffee beans, which have different flavour qualities, the link between the two is not conclusively made. Nor is it clear that the claimed polypeptide would directly or indirectly affect the either the levels of the said storage proteins or the flavour of the resulting beans.

3.2 In order for inventive step to be acknowledged, the Examiner is obliged to satisfy him or herself that the objective technical problem has indeed been solved by the application in suit. As this cannot be said to be the case, inventive step is not acknowledged.

V.4 Requirements for any Amendments Art. 34(2)(b) PCT:

4.1 Any information the applicant may wish to submit concerning the subject-matter of the invention, for example further details of its advantages or of the problem it solves, and for which there is no basis in the application as filed, should be confined to the letter of reply and not be incorporated into the application.

4.2 In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT).

Re Item VII

Certain defects in the international observation

1 The application lacks support from the description. The eight polypeptide sequences disclosed have been designated as cysteine proteinases (SEQ ID NOs. 2 and 16), cysteine proteinase inhibitors (SEQ ID NO. 4, 10, 12 and 16) and two apparently related aspartic endoproteinases (SEQ ID NOs. 6 and 8). However, the application fails to indicate how the function of these proteins were determined in the absence of any biochemical evidence to this end. The application is therefore not considered to fulfill the requirements of Article 5 PCT.

2 The claimed invention is not sufficiently disclosed for the skilled person to be able to modulate coffee flavour precursor levels. The Applicant speculates that introducing the genes provided which encode the eight polypeptides into coffee plants will influence the flavour of the resulting coffee. There is no evidence provided in the application that makes this assumption credible, in fact the description rather indicates that this is uncharted territory.

The Applicant speculates on page 3 that the proteases and protease inhibitors of the application will alter the amino acid and small peptide composition of the beans, but there are apparently no reports directly linking specific levels or ratios of amino acids and high or low flavour qualities. Also no association between these storage protein differences and flavour quality has been noted, and currently no clear evidence exists linking any differences seen for the coffee storage proteins, or other major green bean proteins, and the flavour qualities of coffee.

Against this backdrop, the Applicant provides a number of proteases and protease inhibitors and claims a method for altering coffee flavour without indicating (a) whether these enzymes actually do play a role in flavour development, (b) how the flavour is ultimately altered by these proteins and (c) what promoters to use for the alteration of flavour in terms of tissue specificity, stage specificity and expression levels.

The most that the Applicant can be said to have done is to compare the expression levels of the various proteins between over time and between *C. arabica* and *C. canephora*. However, no correlation is made between expression levels and particular flavour qualities and there is no indication that the differences in flavour over time or between species are caused or influenced by these proteins. Regarding the promoter, the application cites a paper by Leroy *et al.* from 2000 in which coffee is transfected with

a gene under the control of the CaMV promoter, but there do not appear to be any native coffee promoters used in the art.

There is further no worked example teaching the skilled reader how the claimed invention can best be carried out (Rule 5.1(a)(v) PCT). There is clearly a chasm of information between the disclosure of the application and the proposed invention that the skilled person would not be in a position to span without considerable experimentation and inventive energy. The description states on page 5 that "an object of the present invention is to improve the flavour quality of coffee." The application fails to teach the skilled person how to do this and therefore fails to fulfil the requirements of Article 5 PCT.